examined every two hours. As soon as the diagnosis is established, an appendectomy should be performed.

We all realize that patients presenting a generalized peritonitis do not come under the above classification. These patients require observation and preoperative preparation, and when a localized abscess is discovered, it should be drained.

We have stressed the point that the laity must be instructed in the fallacy of delay in consulting a physician, and as to the danger of administering cathartics to patients with abdominal pain. We should also reinstruct the medical profession that immediate surgery is still the treatment of appendicitis.

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H. GLENN BELL, M. D. (University of California Hospital, San Francisco).—It is difficult to discuss a paper when one is in complete accord with the ideas expressed by the author. That is my position in regard to this article. For the past seven years I have followed the plan suggested by Doctor Rhodes and his co-authors, and the results have been very satisfactory.

In one of the tables of Doctor Rhodes' paper he has noted the operative mortality in representative and comparative series from different places. I am familiar with one of the large series cited, and know that in that series, even when treatment has not been delayed, drainage is used much more often than is our custom. Whether that is the entire reason for their higher mortality, it is rather difficult to say. It is reasonable to believe, however, that such drainage plays a tremendous part.

Discussion of isolated cases prove nothing. Only by a careful analysis of a thousand or more cases, such as Doctor Rhodes has made, can one hope to draw conclusions which will help the average surgeon in the more intelligent care of his patients.

In general, the mortality of acute appendicitis in this country is still too high. I am convinced that one reason for this is the feeling that acute appendicitis is a simple disease and that operation for it is simple to perform. Yet we know that appendectomy for acute appendicitis may be a most difficult operation and may tax the surgical skill and judgment of the best surgeon in the country.

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EDWIN M. TAYLOR, M. D. (230 Grand Avenue, Oakland).—It is a pleasure to have an opportunity to discuss some of the controversial points of Doctor Rhodes' paper on "Acute Appendicitis," namely:

When Is the Correct Time to Operate on "Late Cases."—This cannot be set from the beginning of the attack, as some authors attempt to do, but from the physical finding. It seems to me that the very desperately ill, toxic patient, with rapidly diffusing or already diffused peritonitis, has a better chance of recovery if operation is delayed and the so-called Oscher treatment is instituted. The other type of case in which I feel operation should be delayed, is the one with early beginning abscess formation, where there still remains some generalized peritonitis. In the latter type of case, usually in a week or ten days the localized abscess can be drained, and often the appendix removed, if readily accessible, with comparative safety.

Regarding Drains.—The profession in general are using less drains, but I cannot feel quite safe in closing up an abdomen in which the appendix has perforated. I cannot feel that a soft rubber drain is such a hazard in the abdomen. Maybe drains do not actually drain for many hours, but when placed in dependent points, as in the pelvis, in the right lateral gutter or beneath the terminal ileum, are there not sinus tracts formed, through which drainage would take place, should pus accumulate in these areas?

Type of Incision.—This is of paramount importance in treating acute appendicitis with or without peritonitis. The McBurney, or some modification of the lateral incision is certainly the incision of choice when the diagnosis of appendicitis is at all certain. Some large clinics have greatly decreased their mortality by almost universally adopting this type of incision without any change in their use of drains or methods of handling the cases.

Doctor Rhodes' results show a 3.6 per cent operative mortality, certainly lower than any reported from similar clinics.

THROMBOSIS AND EMBOLISM: PREOPERATIVE AND POSTOPERATIVE CARE IN THEIR PREVENTION *

By JOHN H. BREYER, M.D.
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DISCUSSION *by E. Vincent Askey, M. D., Los Angeles; Willard J. Stone. M. D., Pasadena; H. Brodie Stephens, M. D., San Francisco.*

MASSIVE pulmonary embolism has produced tragic and sudden deaths in the practice of every experienced surgeon. It is a postoperative disaster which every surgeon fears. Concerning fatal pulmonary embolism, statistics seem to show that it accounts for about 6 per cent of surgical deaths. One series, by Wharton and Pierson, reported that nearly half of the deaths after gynecologic operations were due to pulmonary embolism. Postoperative thrombophlebitis, which happens more frequently, prolongs hospitalization and often incapacitates the patient for months. Femoral thrombophlebitis, as reported by Albanus, occurred sixty-three times after 1,140 laparotomies, and forty-four of these cases resulted in embolism, of which ten patients died. A critical review of the literature is hereby attempted, hoping to develop some practical plan of prevention which might lessen the incidence.

FREQUENCY

Thrombosis and embolism are complications which occur in many diseases and conditions other than surgical and are, therefore, of general medical interest. They occur in the infectious diseases, as in influenza, pneumonia, tuberculosis, typhoid fever, and in sepsis. They occur in diseases in which the blood itself is altered, as in the anemias, leukemias, and in polycythemia; in cancer and in the degenerative diseases of the vascular system. American, as well as European literature, seems to indicate that the incidence of thrombosis and embolism has increased since the period of the World War. A report from the department of pathology of the University of Toronto, published in 1933, states that they have demonstrated pulmonary emboli in about 10 per cent of routine autopsies upon adults. They found it more common in medical than in surgical cases. In 6,581 necropsies performed in the city hospital at Kiel, Germany, reported in 1934, thrombosis was encountered in 14 per cent, and pulmonary embolism was observed in 9.7 per cent of the autopsies. From 1919 to 1928 the incidence of thrombosis cases had increased eight and one-half times the average for the years prior to 1919. Thrombosis occurred about equally in the two sexes, and was more frequent after forty-five years of age.

RELATION TO SURGICAL TRAUMA

As surgeons we are concerned whether trauma incident to the operation may be an activating factor in the production of thrombosis and embolism. Thrombosis is primarily a physiologic

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process, a defense mechanism, otherwise we would all die of hemorrhage. This defense mechanism comes into action when injury to the blood vessels and tissues takes place. Its initiation appears to be through the action of some vital substance or substances called forth by the injury, which are variously known as fibrin ferment, thromboplastin and thrombokinase, in the formation of which the leukocytes, blood platelets, calcium salts, and tissue extracts play an important rôle. Normally, clot formation takes place sufficient to plug the severed vessels, and then ceases. Why it ceases is as great a mystery as why it starts. When clot formation exceeds its normal required limits and extends into the stream of circulating blood, it becomes the pathologic process of thrombosis. The restraining limiting factors are no longer acting; why, we do not know. Natural control of the tendency to thrombosis may again be reëstablished, holding the tendency in abeyance either permanently or for a time. When again brought under control absorption, organization, endothelialization and canalization of the thrombus may take place.

CONTRIBUTING CAUSATIVE FACTORS OF PATHOLOGIC THROMBOSIS

The most widely accepted contributing factors in the causation of pathologic thrombosis are: (1) slowing of the circulation; (2) changes in the vessel wall due either to trauma or infection; and (3) alterations in the composition of the blood itself. It is now generally recognized that the exciting cause is not one factor alone, but that an interaction of two or more factors is probably necessary for the development of thrombosis.

Slowing of the Circulation. — Slowing of the circulation is probably the most important cause in the development of thrombosis. Thrombosis forms rarely in the heart and arteries, but is comparatively common in the veins. It is rare in the young with efficient circulation, but becomes more frequent in advancing age with degenerative changes in the cardiovascular system, which may result in slowing of the blood stream with lowering of the blood pressure. Thrombosis occurs quite frequently during life in the prostatic plexus of veins. Almost any adult male past middle life has calcified phleboliths in this plexus of veins as a result of previous formation of thrombi, and at death, from any cause, this plexus is likely to be distended with antemortem clots. In the female the cervical and vaginal plexuses may be similarly affected, though less frequently so. A saccular aneurysm is usually more or less filled by a thrombus. It is believed that this is due to the simple stasis or stagnation of the circulating blood at these particular locations. As pointed out by Welch, the slowing and eddying of the circulation causes the platelets to agglutinate along the vessel wall to form a homogeneous layer. Following this there is a settling out of white blood corpuscles, after which fibrin begins to form at the margins of the clot. The vessels most often involved in this type of bland thrombosis are the large veins of the extremities and pelvis, especially the femoral and iliac veins.

Changes in the Vessel Walls.—That changes in the vessel walls due to either trauma or infection may lead to thrombus formation at the site of injury has long been recognized. A thrombosis may develop, however, at a location distant from the injury site and away from the source of infection. Whether a phlebitis is primary, with secondary infection of the thrombus, or vice versa, is difficult to prove. Postoperative and puerperal phlebitides are usually explained as due to infection, being more common in patients in whom infection is present. However, a direct avenue of infection cannot always be demonstrated. Infections no doubt add to the general impairment which increases the tendency to vein complications. Many authors believe that the toxins from infection, necrotic processes, and from tissue damage are probably important contributing factors in promoting thrombosis. The vein reaction to the formation of the thrombus may be a nonbacterial reparative inflammation of the mildest form, or may be of the acutely inflammatory and suppurative type. The thrombus may be but loosely attached to the vein wall or may be firmly attached as a result of the inflammatory reaction.

Alterations in the Composition of the Blood.—Changes in composition of the blood have frequently been described as favoring the development of thrombosis. It is variously attributed to increased friability of the platelets, to increase in their number, to shortening of coagulation time, increased viscosity, accelerated sedimentation rate, dehydration, the augmentation of the clotting factors, and other changes. These are changes which cannot readily be measured, and these elements probably play no rôle in the initiation of the thrombus, but may influence the secondary coagulative phase affecting the propagation of the peripheral portions of the thrombus.

EMBOLI

An embolus, of course, is a solid, oily or gaseous mass in the free blood stream. The danger of thrombo-embolism is inversely proportionate to the amount of vein inflammation accompanying the thrombus. The pathology produced depends on the size of the embolus, its septic or aseptic character, and the size and location of the occluded artery. In pulmonary embolism the right lung is more often involved than the left, and the lower lobes more frequently than other parts of the lung. In order to be of size to obstruct one or more of the main branches of the pulmonary artery, the embolus must take origin from a major vein, which has been shown by autopsy studies in 85 per cent of cases to be usually the femoral or iliac veins. Under such conditions pulmonary edema follows, with death taking place in a few minutes, or within several hours in all but exceptional cases. Fifty per cent die within fifteen minutes.

Moderate-size emboli pass the main pulmonary artery and occlude smaller branches, resulting in pulmonary infarcts. A hemorrhagic consolidation of the infarcted lung or lobe follows, particularly if there is a coexistent respiratory or cardiac impairment. Such infarcts are said to produce a

mortality of 12 to 15 per cent. Many of the smaller-size emboli produce no detectable clinical disturbance, and occur more frequently than is generally supposed. Septic infarcts may result in empyema, pneumonitis or lung abscess. However, the infrequency of infection in pulmonary infarction is offered as evidence of the nonbacterial origin of the thrombus.

CLINICAL OBSERVATIONS ON THROMBOSIS AND EMBOLISM

The following clinical details are of interest in their relation to thrombosis and embolism. Postoperative massive fatal embolism is liable to occur during the convalescent period, usually during the second week, without swelling of the legs or evidence of thrombophlebitis, or any clinical signs which would attract the attention of the surgeon to the likelihood of its occurrence. The majority of the patients are between forty and seventy years of age, and obesity seems to be a predisposing factor. The presence of cardiovascular degenerative changes and carcinoma have been noted in a high percentage of cases. The type of anesthetic used appears to have no bearing on the occurrence of this unfortunate accident which seems to follow abdominal and pelvic operations in about 70 per cent of cases. Fatal embolism most frequently follows stomach surgery, especially for cancer; pelvic surgery, especially for fibroids of the uterus; and operations for prostatic hypertrophy. Gall-bladder surgery, operations on the large intestine, appendectomies and even hernia operations may be followed by this complication. Thyroid surgery is particularly free from postoperative thrombosis and embolism. Thrombophlebitis of the superficial veins of the extremities usually does not result in large emboli, but repeated small infarcts, however, may result from dislodged fragments of the advancing type of phlebitis. When inflammation and thrombosis involve the deep veins of the extremities, it gives the operator concern. Whether such a thrombophlebitis is the suppurative or nonsuppurative type, the formation of an embolus is always a possibility.

PROPHYLAXIS: PREOPERATIVE MEASURES

It has been said, "there is a probability that the tendency to thrombosis may depend upon the patient's physical condition rather than that he has undergone an operation, but it is equally probable that had he not had the operation the thrombosis would not have developed." Prophylaxis against thrombosis and embolism rests largely upon the care with which we estimate our surgical risks, and the preoperative preparation of such impaired cases. This is especially important since the trend is to offer surgery to elderly patients even with late-stage pathology. Preventive treatment aims at (1) prevention of thrombosis and (2) the adequate treatment of manifest thrombosis. The factors responsible for thrombosis have been enumerated. It probably requires the interaction of several factors in the majority of cases, yet some develop thrombosis with a minimum of the contributing factors, and others with the maximum number may escape this complication. The patient should be put in the best possible physical condition and made the best physiologic risk possible under the circumstances. A careful chest examination by an internist should be part of the preoperative routine. Definitely known foci of infection of teeth, tonsils, prostate or cervix should be considered in their possible relation to the inevitable hazard which accompanies any operative procedure. Only the acute surgical emergencies should be operated in the presence of "head colds." The appropriate cardiovascular stimulants should be used when evidence of myocardiac insufficiency is present, such as increased pulse rate with falling blood pressure. Digitalis, a drug much misused by surgeons, is often not only useless, but harmful. The use of digitalis in adequate dosage should be restricted to cardiac insufficiency or decompensation, and especially to disturbances of rhythm such as auricular fibrillation, in which a rapid irregular heart rate is accompanied by a pulse deficit. Slowing of the heart rate in auricular fibrillation as a result of the administration of digitalis is beneficial. The administration of digitalis is of doubtful utility to slow the heart rate in toxic or septic conditions. The use of caffein or the theobromin or theophyllin salts for their general circulatory effects are much more frequently indicated when cardiac stimulation is believed necessary. The body fluids should be increased and maintained by blood transfusions, salt and glucose solutions before, during and after operation. Secondary anemia, if present, should be improved by the various methods available. Starving a patient before operation should be avoided, and when the diet is restricted for any reason the giving of glucose solution is indicated. The work of Mills, Bancroft, and Stanley Brown have demonstrated an increased coagulability of the blood following a high protein diet, while a diet adequate in carbohydrates and fat was not followed by such an effect. It thus seems advisable to place all patients on a low protein diet for the first two weeks following operations within the abdomen or pelvis, in cases of trauma with much tissue mutilation and in cases of phlebitis at all times. Care should be taken to avoid chilling of the patient, before, during, and after operation. The legs and feet must be kept warm, and the local use of heat may be indicated.

Atraumatic surgery is the ideal toward which all surgeons strive. Hemorrhage and shock have a profound influence on venous stasis. The patient's position on the operating table should be made as comfortable as possible, avoiding pressure anywhere which might promote venous stasis, especially in the legs. Harsh and prolonged retraction should not be used and injury to the deep epigastric veins is to be avoided, as thrombosis may start in these vessels. It is better to ligate and divide than to injure veins by puncture, bruis-

ing, or overstretching. Mass ligatures are to be avoided, and sutures penetrating veins should be removed and reapplied. Septic wounds should be thoroughly drained to avoid the spread and absorption of septic products.

POSTOPERATIVE MEASURES

During the postoperative stage nothing should be done which promotes venous stasis, such as use of tight abdominal dressings and binders. When gas distention takes place a binder may become too tight. Due to vomiting and diaphoresis the body fluids may become greatly depleted, which necessitates the use of transfusion of blood, salt and glucose solution. Frequent moving and turning of the patient is indicated. The pain produced by the incision may inhibit the normal respiratory excursion; therefore, encourage deep breathing, as the diaphragm is a circulatory as well as a respiratory organ. The inhalation of carbon dioxid at intervals may be indicated. Moderate elevation of the foot of the bed, provided there are no contraindications, during the first twenty-four hours after operation, when activity is at a minimum, might act as a prophylactic measure favoring return venous flow. It is important to frequently check the blood pressure in order to determine whether the pressure normal for the patient is being maintained. Methods to increase body metabolism may be indicated. Doctor Walters gives two grains of desiccated thyroid gland extract three times a day in all cases except those with increased pulse rate and temperature. The medication is begun two to four days after operation and continued until usually the tenth day. If the pulse and temperature are increased, the use of thyroid extract is discontinued. In the series in which thyroid extract was used the incidence of embolism was reduced to .09 per cent. However, all other known methods of improving the circulation were also instituted. As an effective means in reducing the incidence of thrombosis and embolism some surgeons favor early mobilization of surgical patients. Other surgeons believe that this is done at the expense of wound repair. The adoption of systematic light active and passive exercises provides almost all the advantages gained by a shortened stay in bed without interfering with wound repair. These exercises stimulate peripheral circulation, and add much to the comfort and strength of the patient. Meticulous attention to details and close observation that they are carried out is essential.

When thrombosis of the veins of the legs does occur, statistics show that the results are not frequently fatal. Every case must be decided on its own merits. Thrombosis should be suspected when a low elevation of temperature in a clean surgical case takes place, with increased pulse rate with pain in leg or foot, and with increased size of leg as determined by a tape measure. The greatest possible caution is called for to prevent dislodging emboli. This necessitates the gentlest possible handling during nursing care, particularly during the bath and in the use of the bed pan. The leg should be kept elevated on a pillow to keep it warm, and to avoid pressure of the bed clothes. Whatever seemed necessary in the prevention of thrombosis is now reversed for the prevention of embolism. The guiding principle is rest until the thrombus has been anchored by organization. Occurrence of pleuritic pains in the postoperative period should be considered the result of infarction and not a "dry pleurisy," and proper precautions should be instituted.

IN CONCLUSION

The important thing is to recognize the possibility, and even probability, of the development of thrombosis or embolism, and to endeavor to find some practical method for actively combating their development. To recognize a complication at an early stage makes treatment more effective.

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DISCUSSION

E. VINCENT ASKEY, M. D. (1930 Wilshire Boulevard, Los Angeles).—Doctor Breyer has summed up very capably the problem which confronts us. He has called to our attention work which has been done on this subject. He has adequately discussed those factors involved in the physical forces predisposing to thrombosis, namely, trauma, infection, stasis or slowing of the circulation, and dehydration.

However, if I understood him correctly, he stated that changes in blood composition cannot be measured readily and that these elements probably play no part in the initiation of a thrombus, but only with the propagation of a thrombus already started. This, I think, is not strictly so, and we differ in this opinion. There have been methods suggested to warn us of impending thrombosis and definite treatments proposed to combat it.

It is probable that in the future more attention will be paid to the biochemical changes found in the blood itself in thrombosis and embolism.

Bancroft and Stanley-Brown have suggested an index of clotting potentiality. This index can be determined in any well-equipped hospital laboratory. They believe it is of as great value in denoting impending thrombosis as is a leukocyte count in an infection. In cases showing a high index of clotting potentiality they advise the repeated use of 10 per cent solution of sodium thiosulphate intravenously. They believe their results show definite value. The effectiveness of the sodium thiosulphate they explain as follows:

In the body tissues there is an oxidation enzyme called glucothione—a sulphur compound. Its action is to lessen the development of lactic acid. Lactic acid results from injury to tissues. Sodium thiosulphate supplies sulphur, which increases the content of glucothione either by neutralization of the lactic acid or by the formation of new glucothione.

Howell has prepared, from dogs' livers, an anticoagulant called Heparin and has used it in thrombosis cases with good results. Rowntree, however, warns us that as yet preparations available are not pure enough to be used without danger.

Recently De Takats has called attention to the marked amelioration of symptoms in cases of frank embolism by the use of papaverine. This drug relieves surrounding vasoconstriction and spasm, and encourages early collateral circulation.

The problem is as yet unsolved. Thrombosis and embolism as postoperative complications constitute a fearsome tragedy in the practice of any surgeon.

WILLARD J. STONE, M. D. (65 North Madison Avenue, Pasadena).—Every physician who has witnessed the sudden and, in many instances, unexpected death of a patient from pulmonary embolism during convalescence from a surgical operation, will appreciate Doctor Breyer's thoughtful review of the methods which may be used to prevent such a tragic accident.

From the medical standpoint, the circulatory condition of obese patients with slow heart rates and subnormal metabolism may be improved by the administration of thyroid extract during the first ten days of convalescence. Likewise the administration of glycocoll, for its general stimulating action on metabolism, leading to improved circulatory efficiency, may be helpful. The routine use of digitalis during convalescence whenever the heart rate is increased as a result of surgical shock, is difficult to justify from a pharmacologic standpoint. Its use should be limited, as Doctor Breyer has emphasized, to the treatment of auricular brillation and cardiac decompensation. With other forms of cardiac insufficiency, especially if the heart rate is slowed, the administration of theobromin or theophyllin salts may be beneficial. Strychnin as a circulatory tonic has gradually become less popular because of the insufficient dosage commonly employed. For patients with slow heart rates who may be believed to be unusually susceptible to thrombotic tendencies, the dosage should approximate one-tenth grain four times daily.

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H. BRODIE STEPHENS, M. D. (384 Post Street, San Francisco).—Doctor Breyer has presented an excellent review of the distressing complication of postoperative thrombosis and embolism. The author in his review rightly emphasizes the frequency of this complication and, because of its frequency, a discussion of the subject is timely.

A careful study and adherence to the principles laid down by Doctor Breyer will undoubtedly frequently prevent this complication; or, if thrombosis does occur, its seriousness will be lessened by adopting the general principles set up by the author.

Notwithstanding the thoroughness of this paper, all will agree, I believe, that there will be still many cases of postoperative thrombosis and even embolism, in spite of whatever we do before or after operation. It appears to me that we have far from solved the problem, and much experimentation and clinical investigation are still needed, if we are to thoroughly understand and prevent this complication so far as it concerns the surgical patient.

Pulmonary embolism has been satisfactorily handled by immediate operation, but it requires a well-trained house staff and a sterile set-up for such an emergency. We have felt this to be a worthwhile routine, and the house staffs are each year given instruction in how to handle this emergency.

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DOCTOR BREYER (Closing).—I appreciate the suggestions made by the discussers and the emphasis placed on certain phases of the subject, which I could not stress due to the shortness of the paper. The underlying surgical principle of the recommended exercises in the prevention of thrombosis is the effect of muscle action on the venous circulation. We all have made use of this principle, when we ask the donor for a blood transfusion, to open and shut his hand after the trochar needle has been inserted in the median basilic vein.

Dr. Alvin G. Foord, pathologist at our Huntington Memorial Hospital, has stated to me that when the autopsy surgeon of the pathologic service of Dr. Julius Erdheim of Vienna failed to find the source of the pulmonary embolism, Doctor Erdheim would ask if the veins of the calves of the legs had been dissected out and explored. Very often when this was done, the origin of the embolus was found to be there.

Exercises that will cause tensing of the flexors and extensors of the foot, and of the tensor femoris muscle, will produce the desired effect.

WHOOPING COUGH: ITS PROPHYLAXIS AND TREATMENT*

By J. M. FRAWLEY, M.D.
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DISCUSSION *by A. J. Scott, M. D., Los Angeles; Francis Scott Smyth, M. D., San Francisco; Charlotte Singer Brooks, M. D., San Francisco; Edward B. Shaw, M. D., San Francisco.*

A PROGRAM directed toward the control of whooping cough has been carried on since 1932 in the public schools of Fresno. Two methods have been employed, viz., isolation and vaccination.

ISOLATION

In order to be of benefit as a factor in controlling the spread of whooping cough in the classroom, isolation has to begin at the onset of the cough. This is possible only when the cough-plate method [1] is used to detect early cases.

At the outset of this study, cough plates were made for us by Doctor Krueger at the bacteriological department of the University of California in Berkeley. They were always received in perfect condition, and the exposed plates were returned by mail after incubating overnight. During one school season they were sent to us by Doctor Kellogg from the State Department of Public Health Laboratories in Berkeley, but two years ago a cough-plate station was organized at the Fresno County Hospital, and the plates have been made and read there. They are prepared according to a method recommended by J. J. Miller.

TECHNIQUE

The technique is described in detail by Kendrick, Miller, and Lawson [2] in the American Public Health Association Year Book for 1935-1936:

A modification of Bordet and Gengou's potato blood agar is made as follows:

Base:
Peeled sliced potatoes, 500 mg.
Glycerin, U. S. P., 40 cc.
Distilled water, 1,000 cc.

Boil the potatoes in glycerin and water until soft. Make up to volume, strain through gauze and allow to stand for sedimentation. Siphon off the supernatant liquid.

To 500 cubic centimeters of clear potato extract add:
Sodium chlorid solution, 0.75 per cent, 1,500 cc.
Agar, Bacto, 60 gm.
Proteose Peptone, 20 gm.

Let stand for fifteen minutes, to saturate the agar. Heat until the agar is dissolved, and dispense in amounts convenient for storage. Autoclave for twenty-five minutes at fifteen pounds pressure (120 degrees centigrade). This base may be stored indefinitely.

Finished medium—To the melted base, at 45 degrees centigrade, add blood to make a final concentration of at least 15 per cent. The blood should be used when fresh, never more than seventy-two hours after it has been obtained. It may be from sheep, horse, or human source, but avoidance of horse blood for the vaccine medium is recommended. Mix the blood with the base by whirling, and pour into plates—about 15 cubic centimeters per Petri plate. Either glass plates or metal boxes may be used.

The potato extract-glycerin-agar base is kept stored in the ice-box, and sufficient Petri plates for a week's supply are made up by adding blood

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